

In addition, a typographical error has been corrected at page 36, line 1, in which the text was changed to read "Table 2" instead of "Table 1." It is self-evident from the context that the applicant had intended to refer to Table 2, not Table 1. For example, the text at page 36, line 1 reads "Table 1 below," and the only table located "below" is Table 2.

The specification has been amended to change "SEQ ID NO:10" to "SEQ ID NO:11" in instances where the text refers to murine RANKL polypeptide. Since SEQ ID NO:10 discloses the same polypeptide sequence as SEQ ID NO:11, this amendment does not constitute the addition of new matter to the application.

The specification has been amended also to update the status of parent applications recited in the priority statement. This update does not constitute the addition of new matter to the specification.

New claims 68-90 have been added to the application and are supported throughout the specification, for example, as indicated in the table shown below. This table furthermore indicates the correspondence between each of claims 68-90 and claims present in U.S. Patent No. 6,242,586, from which new claims 68-90 were copied. A copy of U.S. 6,242,586 is provided in conjunction with the Information Disclosure Statement submitted herewith. Support for the invention described in the new claims also can be found in U.S. patent application No. 60/059,978, filed December 22, 1996, which is the earliest-filed patent application from which the present application claims the benefit of priority.

<b>Claim no.</b>	<b>Corresponding claim in US 6,242,586<sup>†</sup></b>	<b>Support in specification of 09/688,459</b>
68	7	pg 13, lines 12-19; pg 25, line 18 to pg 26, line 3
69	8	pg 15, line 33 to pg 17, line 11
70	9	pg 15, line 33 to pg 17, line 11
71	10	pg 12, lines 15-17
72	11	pg 10, lines 30-36
73	12	pg 10, lines 30-36
74	12	pg 10, lines 30-36
75	21	pg 27, line 26 to page 28, line 1
76	23	pg 13, lines 15-19; pg 25, line 17 to pg 26, line 3
77	24	pg 13, line 12 to pg 15, line 26
78	26	pg 11, lines 24-26; pg 13, lines 12-15; pg 25, line 18 to pg 26, line 3
79	27	pg 11, lines 17-26
80	28	pg 15, lines 16-18; pg 16, line 26 to pg 17, line 11

<sup>†</sup> Claims 1, 3-6, 13 and 17-19 have been copied from U.S. 6,242,586 in related patent application no. 09/687,809.

Claim no.	Corresponding claim in US 6,242,586 <sup>†</sup>	Support in specification of 09/688,459
81	29	pg 15, lines 16-18; pg 16, line 26 to pg 17, line 11
82	30	pg 15, lines 16-18; pg 16, line 26 to pg 17, line 11
83	31	pg 15, lines 16-18; pg 16, line 26 to pg 17, line 11
84	32	pg 12, lines 15-17
85	33	pg 12, lines 15-17
86	34	pg 13, lines 12-15; pg 15, line 34 to pg 16, line 2; pg 17, line 19 to pg 18, line 30
87	35	pg 13, lines 12-15; pg 15, line 34 to pg 16, line 2; pg 17, line 19 to pg 18, line 30
88	36	pg 13, lines 12-15; pg 15, line 34 to pg 16, line 2; pg 17, line 19 to pg 18, line 30
89	37	pg 10, lines 30-36
90	38	pg 11, lines 23-26 and 31-32

Applicants are considering whether to file a formal request for an interference with U.S. 6,242,586 with regards to new claims 68-90. If after an appropriate analysis an interference is deemed appropriate, such a request will be filed in due course.

In accord with the information presented above, new claims 68-90 do not constitute the addition of new matter to the application.

#### Objections to the Specification

The examiner has objected to the specification because in several instances "SEQ ID NO:10" is recited in reference to a polypeptide sequence. Since SEQ ID NO:10 discloses both a nucleic acid and polypeptide sequence, the applicants believe that no correction should be necessary. However, to accommodate the examiner's preference, the specification in such instances has been amended as shown above to read "SEQ ID NO:11" instead of "SEQ ID NO:10" or to read "SEQ ID NO:13" instead of "SEQ ID NO:12." Accordingly, the examiner is asked to remove this ground for objection to the specification.

#### Priority

The initial paragraph of the specification, which describes priority applications, has been updated to reflect the current status of the parent applications. These updates do not constitute the addition of new matter to the application.

#### Rejections under 35 U.S.C. § 112, first paragraph

Claims 37, 41, 45, 49, 53, 57, 61 and 65 have been rejected under 35 U.S.C. § 112, first paragraph, in view of the examiner's assertion that the specification lacks any description of a polypeptide corresponding to

positions 119-294 of SEQ ID NO:11. Such a polypeptide is described in the specification at page 33, lines 29-32. It is self-evident from SEQ ID NO:10 that nucleotides 357-884 (recited in claim 41), encode this polypeptide. Accordingly, the examiner is respectfully requested to withdraw this ground for rejection.

#### Patent Drawing Review

The Draftsperson has objected to the quality of Figures 1-3 and has required that new drawings be submitted. This requirement is moot with respect to Figure 2 as this drawing has been cancelled from the application. To accommodate the cancellation of Figure 2, Figure 3 has been renumbered by amendment so that its caption reads "Figure 2" instead of "Figure 3." A reply to the Draftsperson's Review is attached, together with new copies of Figures 1 and 2 (formerly "Figure 3") that correct the deficiencies noted in the Draftsperson's Report.

#### **CONCLUSIONS**

Claims 36-90 are now pending in the application and in view of the above remarks are believed to be in condition for allowance. As noted above, the applicants at a later date may request an interference with U.S. 6,242,586. If any further questions remain in the application, the examiner is asked to contact the undersigned at her direct dial number given below.

Respectfully submitted,



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Attachments:

Marked-up copy of Figure 3;  
Substitute version of Figure 3.

#### CERTIFICATE OF MAILING

I hereby certify that this Amendment, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service as first-class mail under 37 CFR §1.8 on the date indicated below, and is addressed to: Commissioner for Patents, Washington, DC 20231.

Date: April 10, 2002

By: 

**Appendix to Amendment filed April 18, 2002**

(marked up version of amendments requested by the attached Amendment)

**Cross-Reference to Related Patent Applications:**

-- This application is a divisional of [co-pending] U.S. patent application 08/995,659, filed December 22, 1997, now U.S. patent 6,242,213, which [is a continuation-in-part of] claims benefit of priority under 35 U.S.C. §119(e) from USSN 60/064,671, filed October 14, 1997, USSN 60/077,181, [and a continuation in part of 08/813,509,] filed March 7, 1997, and [a continuation-in part of] USSN 60/059,978, filed December 23, 1996. --

**Specification:**

Page 3, lines 4-8:

-- Figure 2 [Figure 3] demonstrates that RANKL enhances DC allo-stimulatory capacity. Allogeneic T cells were incubated with varying numbers of irradiated DC cultured as described in Example 13. The cultures were pulsed with [<sup>3</sup>H]-thymidine and the cells harvested onto glass fiber sheets for counting. Values represent the ± standard deviation (SD) of triplicate cultures. --

Page 5, lines 10-19:

-- Soluble RANKL comprises a signal peptide and the extracellular domain or a fragment thereof. An exemplary signal peptide is that shown in SEQ ID NO:9; other signal (or leader) peptides are well-known in the art, and include that of murine Interleukin-7 or human growth hormone. RANKL is similar to other members of the TNF family in having a region of amino acids between the transmembrane domain and the receptor binding region that does not appear to be required for biological activity; this is referred to as a 'spacer' region. Amino acid sequence alignment indicates that the receptor binding region is from about amino acid 162 of human RANKL to about amino acid 317 (corresponding to amino acid 139 through 294 of murine RANKL, SEQ ID NO:11 [SEQ ID NO:10]), beginning with an Ala residue that is conserved among many members of the family (amino acid 162 of SEQ ID NO:13 [SEQ ID NO:12]). --

Page-5, lines 20-34:

-- Moreover, fragments of the extracellular domain will also provide soluble forms of RANKL. Those skilled in the art will recognize that the actual receptor binding region may be different than that predicted by computer analysis. Thus, the N-terminal amino acid of a soluble RANKL is expected to be within about five amino acids on either side of the conserved Ala residue. Alternatively, all or a portion of the spacer region may be included at the N-terminus of a soluble RANKL, as may be all or a portion of the transmembrane and/or intracellular domains, provided that the resulting soluble RANKL is not membrane-associated. Accordingly, a soluble RANKL will have an N-terminal amino acid selected from the group consisting of amino acids 1 through 162 of SEQ ID NO:13 [SEQ ID NO:12] (1 through 139 of SEQ ID NO:11 [SEQ ID NO:10]). Preferably, the amino terminal amino acid is between amino acids 69 and 162 of SEQ ID NO:13 [SEQ ID NO:12] (human RANKL; amino acids 48 and 139 of SEQ ID NO:11 [SEQ ID NO:10]). Similarly, the carboxy terminal amino acid can be between amino acid 313 and 317 of SEQ ID NO:13 [SEQ ID NO:12] (human RANKL; corresponding to amino acids 290 through 294 of SEQ ID NO:11 [SEQ ID NO:10]). Those skilled in the art can prepare these and additional soluble forms through routine experimentation. --

Page 25, line 29 to page 26, line 3:

-- A clone encoding a protein that specifically bound RANK was isolated and sequenced; the clone was referred to as 11H. An expression vector containing murine RANKL sequence, designated pDC406:muRANK-L (in *E. coli* DH10B), was deposited with the American Type Culture Collection, Manassas, VA [Rockville, MD] (ATCC) on December 20, 1996, under terms of the Budapest Treaty, and given accession number 98284. The nucleotide sequence and predicted amino acid sequence of this clone are illustrated in SEQ ID NO:10. This clone did not contain an initiator methionine; additional, full-length clones were obtained from a 7B9 library (prepared substantially as described in US patent 5,599,905, issued February 4, 1997); the 5' region was found to be identical to that of human RANKL as shown in SEQ ID NO:13 [SEQ ID NO:12], amino acids 1 through 22, except for substitution of a Gly for a Thr at residue 9. --

Page 30, line 36 to page 31, line 10:

-- Addition of RANKL to DC cultures significantly increased the degree of DC aggregation and cluster formation above control cultures, similar to the effects seen with CD40L [(Figure 2)]. Sorted human CD1a<sup>+</sup> DC were cultured in a cytokine cocktail (GM-CSF, IL-4, TNF- $\alpha$  and FL) [(upper left panel)], in cocktail plus CD40L (1 $\mu$ g/ml) [(upper right)], in cocktail plus RANKL (1 $\mu$ g/ml) [(lower left)], or in cocktail plus heat inactivated ( $\Delta$ H) RANKL (1 $\mu$ g/ml) [(lower right)] in 24-well flat bottomed culture plates

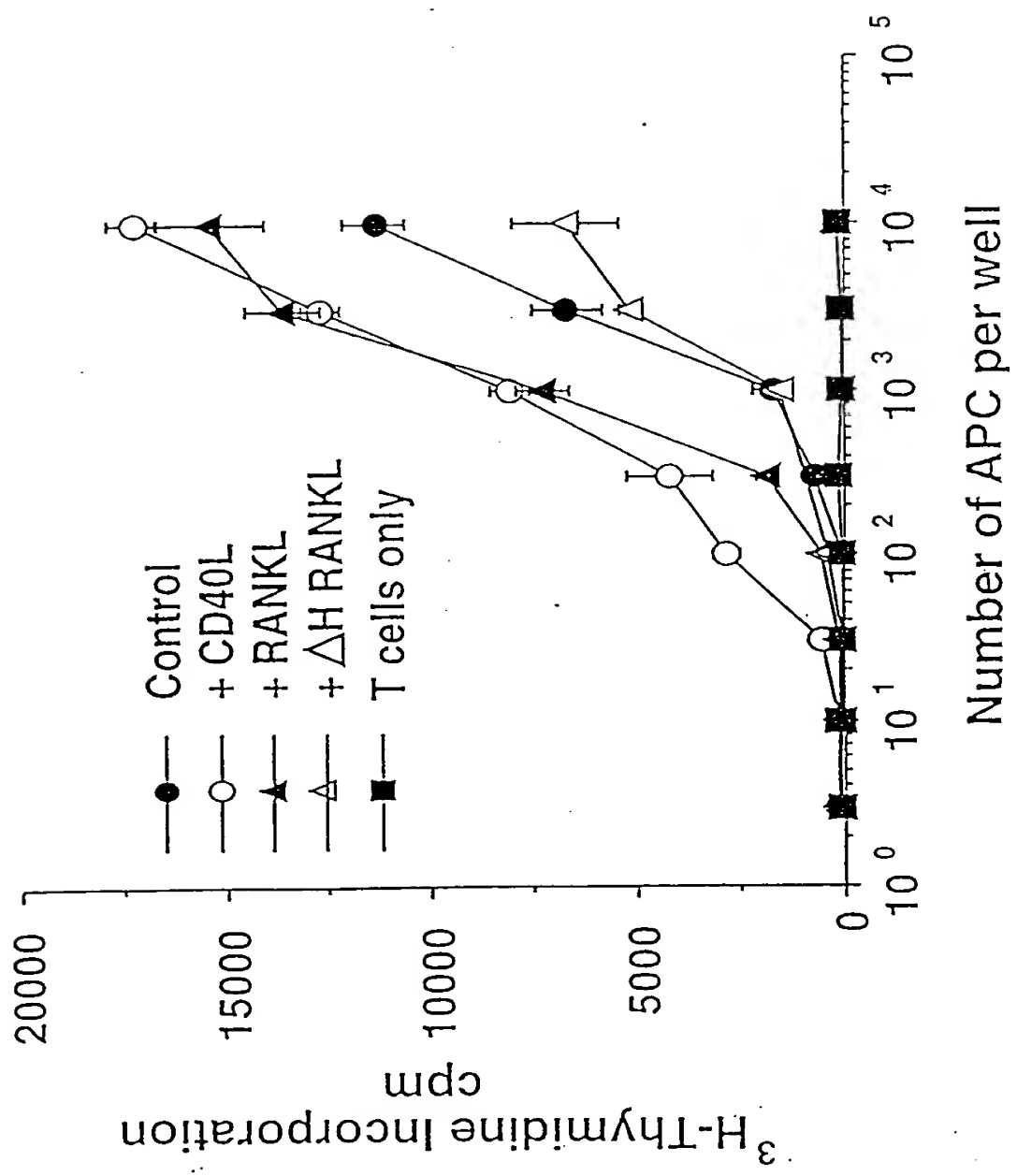
in 1 ml culture media for 48-72 hours and then photographed using an inversion microscope. An increase in DC aggregation and cluster formation above control cultures was not evident when heat inactivated RANKL was used, indicating that this effect was dependent on biologically active protein. However, initial phenotypic analysis of adhesion molecule expression indicated that RANKL-induced clustering was not due to increased levels of CD2, CD11a, CD54 or CD58. --

Page 31, lines 11-24:

-- The addition of RANKL to CD1a<sup>+</sup> DC enhanced their allo-stimulatory capacity in a mixed lymphocyte reaction (MLR) by at least 3- to 10-fold, comparable to CD40L-cultured DC (Figure 2) [(Figure 3)]. Allogeneic T cells (1x10<sup>5</sup>) were incubated with varying numbers of irradiated (2000 rad) DC cultured as indicated above [for Figure 2] in 96-well round bottomed culture plates in 0.2 ml culture medium for four days. The cultures were pulsed with 0.5 mCi [<sup>3</sup>H]-thymidine for eight hours and the cells harvested onto glass fiber sheets for counting on a gas phase  $\beta$  counter. The background counts for either T cells or DC cultured alone were <100 cpm. Values represent the mean  $\pm$  SD of triplicate cultures. Heat inactivated RANKL had no effect. DC allo-stimulatory activity was not further enhanced when RANKL and CD40L were used in combination, possibly due to DC functional capacity having reached a maximal level with either cytokine alone. Neither RANKL nor CD40L enhanced the *in vitro* growth of DC over the three day culture period. Unlike CD40L, RANKL did not significantly increase the levels of HLA-DR expression nor the expression of CD80 or CD86. --

Page 35, line 34 to page 36, line 2:

-- Comparison of the nucleotide sequence of murine and human RANK indicated that there were several conserved regions that could be important for TRAF binding. Accordingly, a PCR-based technique was developed to facilitate preparation of various C-terminal truncations that would retain the conserved regions. PCR primers were designed to introduce a stop codon and restriction enzyme site at selected points, yielding the truncations described in Table 2 [Table 1] below. Sequencing confirmed that no undesired mutations had been introduced in the constructs. --



~~Figure 3~~  
Figure 2

3/6  
2/2